Selenium and sex steroid hormones in a US nationally representative sample of men: A role for the link between selenium and estradiol in prostate carcinogenesis?

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Abstract: BACKGROUND Given the recent findings from pooled studies about a potential inverse association between selenium levels and prostate cancer risk, the current cross-sectional study aimed to investigate the association between serum selenium and serum concentrations of sex steroid hormones including estradiol in a nationally representative sample of US men to investigate one mechanism by which selenium may influence prostate cancer risk. METHODS The study included 1,420 men aged 20 years or older who participated in the Third National Health and Nutrition Examination Survey (NHANES III) between 1988 and 1994. We calculated age/race-ethnicity-adjusted and multivariable-adjusted geometric mean serum concentrations of total and estimated free testosterone and estradiol, androstanediol glucuronide (AAG), and sex hormone binding globulin (SHBG) and compared them across quartiles of serum selenium. RESULTS Adjusting for age, race/ethnicity, smoking status, serum cotinine, household income, physical activity, alcohol consumption and percent body fat, mean total estradiol (e.g. Q1:38.00 pg/mL (95%CI:36.03-40.08) vs Q4:35.29 pg/mL (33.53-37.14); Ptrend=0.050)and free estradiol [e.g.Q1: 0.96 pg/mL (95%CI: 0.92-1.01) vs Q4: 0.90 (95%CI:0.85-0.95); Ptrend=0.065] concentrations decreased over quartiles of selenium. Stratification by smoking and alcohol consumption, showed that the latter observation was stronger for never smokers (Pinteraction=0.073) and those with limited alcohol intake (Pinteraction=0.017). No associations were observed for the other sex steroid hormones studied. CONCLUSION Our findings suggests that a possible mechanism by which selenium may be protective for prostate cancer is related to estrogen. IMPACT Further studies of longitudinal measurements of serum and toenail selenium in relation to serum measurements of sex steroid hormones are needed.

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Selenium and sex steroid hormones in a US nationally representative sample of men: A role for the link between selenium and estradiol in prostate carcinogenesis?

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Abstract

Background: Given the recent findings from pooled studies about a potential inverse association between selenium levels and prostate cancer risk, the current cross-sectional study aimed to investigate the association between serum selenium and serum concentrations of sex steroid hormones including estradiol in a nationally representative sample of US men to investigate one mechanism by which selenium may influence prostate cancer risk.

Methods: The study included 1,420 men aged 20 years or older who participated in the Third National Health and Nutrition Examination Survey (NHANES III) between 1988 and 1994. We calculated age/race-ethnicity-adjusted and multivariable-adjusted geometric mean serum concentrations of total and estimated free testosterone and estradiol, androstanediol glucuronide (AAG), and sex hormone binding globulin (SHBG) and compared them across quartiles of serum selenium.

Results: Adjusting for age, race/ethnicity, smoking status, serum cotinine, household income, physical activity, alcohol consumption and percent body fat, mean total estradiol (e.g. Q1: 38.00 pg/mL (95%CI: 36.03-40.08) vs Q4: 35.29 pg/mL (33.53-37.14); P_{trend}=0.050)and free estradiol [e.g. Q1: 0.96 pg/mL (95%CI: 0.92-1.01) vs Q4: 0.90 (95%CI: 0.85-0.95); P_{trend}=0.065] concentrations decreased over quartiles of selenium. Stratification by smoking and alcohol consumption, showed that the latter observation was stronger for never smokers ($P_{interaction}$=0.073) and those with limited alcohol intake ($P_{interaction}$=0.017). No associations were observed for the other sex steroid hormones studied.

Conclusion: Our findings suggests that a possible mechanism by which selenium may be protective for prostate cancer is related to estrogen.

Impact: Further studies of longitudinal measurements of serum and toenail selenium in relation to serum measurements of sex steroid hormones are needed.

Introduction

A recent Cochrane review indicated that findings for the potential anti-tumourigenic effects of selenium are inconsistent (1). In the context of prostate cancer, they identified 17 epidemiological studies, for which the summary OR was 0.79 (95%CI: 0.69-0.90) when comparing higher selenium exposure to lower. When stratifying by method of selenium assessment, an inverse association with prostate cancer risk was observed for higher baseline biomarkers (OR: 0.76; 95%CI: 0.67-0.88), but not for higher estimated dietary selenium intake (OR: 1.00; 95%CI: 0.73-1.36). Among the selenium biomarkers, the inverse association was stronger for toenail (OR: 0.53; 95%CI: 0.35-0.81) than blood (OR: 0.82; 95%CI: 0.72-0.93) levels (1). Recent findings of the Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group, which pooled primary data from prospective studies, corroborated this observation, noting that toenail selenium was inversely associated with total prostate cancer (OR: 0.29; 95%CI: 0.22-0.40), but not blood selenium (2).

Some randomized controlled trials (RCT) have also observed beneficial effects of selenium supplements on prostate cancer risk as a secondary outcome (3, 4). In an RCT of 974 men with a history of either basal call or squamous cell carcinoma who were randomized to either a daily supplement of 200 µg of selenium or placebo, Clarke *et al*. showed that selenium was associated with 63% reduction in prostate cancer risk (p=0.002), but not the primary outcome of recurrent skin cancer (4). A secondary analysis of this RCT observed that significant reductions in prostate cancer risk were only observed for those with selenium concentrations <123.3 ng/mL (lowest two tertiles) (5). In contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT), a large RCT designed specifically to investigate selenium supplementation in the prevention of prostate cancer, did not show a benefit (6), including in men with lower baseline toenail selenium status (7).

The biological mechanisms underlying a potential protective effect of selenium for cancer are not well understood. One mechanism suggested to explain a link between selenium and prostate cancer,

is genetic variation in selenoproteins and related antioxidant enzymes (8). Another more recently suggested hypothesis is focused on a link with sex steroid hormones. For example, even though estrogen therapy was historically used to decrease androgen levels of men with advanced prostate cancer, recent studies suggest a role in prostate carcinogenesis. Estrogen has been shown to induce neoplastic epithelial morphology in both human and rat prostates and to regulate prostate specific gene expression. Anti-estrogens have also been shown to inhibit development and progression of prostate cancer under experimental and clinical conditions (9).

Given the growing evidence for a role of estrogen in prostate cancer carcinogenesis (10), it is of interest to also investigate selenium action in relation to prostate cancer risk. For example, it is hypothesized that the progressive emergence of the ERalpha during prostate cancer progression and hormone refractory disease presents a mechanism through which the tumour can bypass androgens using estrogen and progesterone. Nevertheless, few studies have investigated the link between selenium and other sex steroid hormones such as testosterone and sex hormone-binding globulin (SHBG); no clear associations have been observed to date (11, 12).

If underlying biological mechanisms were better understood, they may inform the current inconsistency among studies and trials addressing selenium and risk of cancer, especially prostate cancer. Hence, in the current cross-sectional study we investigated the association between serum selenium and serum concentrations of sex steroid hormones including estradiol in a nationally representative sample of US men to inform one mechanism by which selenium may influence prostate cancer risk.

Methods

Study population

NHANES III was conducted by the National Center for Health Statistics (NCHS) between 1988 and 1994 (13) and designed as a multistage stratified, clustered probability sample of the US civilian non-institutionalized population who was at least two months old. All participants were interviewed at home and underwent an extensive physical examination, including a blood sample performed at a mobile examination center (13). NHANES III was conducted in two phases (1988-1991 and 1991-1994) which both lead to independent unbiased national estimates of health and nutrition characteristics. Within each phase, participants were randomly assigned to enter either the morning or afternoon/evening examination session. Of the 2,205 men, who took part in the morning session of Phase I (1988-1991), we selected all men aged 20+ years with no history of prostate cancer, who had serum total testosterone, total estradiol, SHBG, androstanediol glucuronide (AAG), as well serum selenium measured (n=1,420).

Hormone measurements

Sex steroid hormones were assayed using stored serum samples at Children's Hospital Boston, MA. Competitive electrochemiluminescence immunoassays on the 2010 Elecsys autoanalyzer (Roche Diagnostics, Indianapolis, IN) were used to measure testosterone, estradiol, and SHBG concentrations in 2005. AAG, an indicator of the conversion of testosterone to dihydrotestosterone, was assessed with an enzyme immunoassay (Diagnostic Systems Laboratories, Webster, TX). Laboratory technicians were blinded to participant characteristics. These assays had the following detection limits for testosterone, estradiol, AAG, and SHBG: 0.02 ng/mL, 5 pg/mL, 0.33 ng/mL, and 3 nmol/L, respectively. The coefficients of variation for quality control specimens were measured for two or three concentrations each: 5.9% and 5.8% at 2.5 and 5.5 ng/ml for testosterone; 2.5%, 6.5%, and 6.7% at 39.4, 102.7 and 474.1 pg/mL for estradiol; 9.5% and 5.0% at 2.9 and 10.1 ng/mL for AAG; and 5.3% and 5.9% at 5.3 and 16.6 nmol/L for SHBG. The interassay coefficient of variation for quality control samples with a mean estradiol concentration of 39.4 pg/nL (within the typical range for adults males) was 2.5% (14). Predefined formulas were used to estimate free testosterone and

estradiol using each man's total testosterone and SHBG concentrations, along with the population normal albumin concentration in men (15, 16).

Exposure measurements and covariates

Serum selenium concentrations were measured in venous blood samples taken during the NHANES examinations using atomic absorption spectrometry (17). Information on age, race/ethnicity, income, cigarette smoking, and physical activity was collected by interview. The following activities were used to define vigorous physical activity: jogging or running; swimming or aerobics (for men 40 years or older); biking, dancing, gardening, and calisthenics (for men 65 years or older); and walking and lifting weights (for men 80 years and older). Frequency of alcohol consumption was measured by a food frequency questionnaire and categorized by times per week. Percent body fat was estimated from anthropometric and bioelectrical impedance data using the equations of Chumlea and colleagues (18). Serum cotinine levels were measured as previously described (19).

The protocols for the conduct of NHANES III were approved by the Institutional Review Board of the NCHS, Centers for Disease Control and Prevention. All participants provided written informed consent. The assay of stored serum specimens for the Hormone Demonstration Program was approved by the Institutional Review Boards at the Johns Hopkins Bloomberg School of Public Health and the NCHS, Centers for Disease Control and Prevention.

Statistical Analysis

We used phase I morning sampling weights for NHANES III to account for sampling variability and adjust for differential probability of selection of persons (13). Age-adjusted means or percentages of characteristics of the men by quartiles of serum selenium concentration were calculated after adjusting for the age distribution of the US population according to the 2000 Census. Next, we calculated adjusted geometric mean concentrations of the sex steroid hormones and their 95%

confidence intervals (CI) by quartiles of serum selenium using linear regression. As the hormone concentrations were not normally distributed, we used log-transformations. Multivariable models was adjusted for age (continuous), race/ethnicity, and factors that have been associated with hormone concentrations in previous NHANES III analyses: cigarette smoking (never, former, current), serum cotinine (continuous), household income (<$20,000 and ≥$20,000), vigorous physical activity (yes or no), alcohol intake (0, <2, 2-3, 4-6 times a week, or daily or more), and percent body fat (continuous).

For those sex steroid hormones for which the multivariable models indicated a trend across selenium quartiles, we conducted further stratified analyses. Specifically, we assessed whether age (20-40, 40-60, and 60+ years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other), smoking status (never, former, current), or alcohol consumption (never, <2, 2+ drinks per week) modified the association between serum selenium and sex steroid hormones. Given the strong potential for confounding by smoking and alcohol drinking, we investigated the association between sex steroid hormones and selenium within never smokers and those with limited alcohol consumption (<2 drinks per week). Interaction was assessed by adding an interaction term and testing its coefficient using the Wald test.

All statistical analyses were conducted with SAS release 9.2 (SAS Institute, Cary, NC) and SUDAAN 9.0 software (Research Triangle Park, NC) as implemented in SAS 9.2.

Results

In this sample of men 20+ years old in NHANES III, those with selenium levels ≥122 ng/mL (i.e., third and fourth quartile) were more likely to be non-Hispanic white, to be never smokers, and to participate in vigorous physical activity (only those with selenium levels ≥133 ng/mL), as compared to those with selenium levels <122 ng/mL **(Table 1)**. More specifically, those with selenium levels

<122 ng/mL were more likely to be non-Hispanic black, to be smokers, to have lower household income, to have a higher percent body fat, but drink fewer alcoholic drinks **(Table 1)**.

Adjusting for age and race/ethnicity, mean total and free estradiol concentrations were statistically significantly lower in men with higher selenium concentrations **(Table 2)**. These differences in total and free estradiol levels were attenuated, but remained statistically significant after further adjustment for smoking status, cotinine levels, alcohol consumption, physical activity, income, and percent body fat. This inverse association was also observed for the estradiol/total testosterone ratio, but no associations were observed for total or free testosterone, AAG, and SHBG and serum selenium in these multivariate models **(Table 2)**.

Estradiol decreased across quartiles of serum selenium in both non-Hispanic black and white **(Table 3)**. However, this trend was not statistically significant, and also less obvious for Mexican-American men and men of other race/ethnicities. The inverse association between estradiol and selenium was only apparent among never smokers ($P_{interaction}$=0.073), and those who drank <2 alcoholic drinks per week ($P_{interaction}$= 0.017). Additionally, the inverse association between estradiol and selenium was most notable in those age 60+ years ($P_{interaction}$=0.618).

Discussion

In this nationally representative sample of US men, we observed that mean total estradiol and free estradiol concentrations decreased over quartiles of selenium. Stratification by smoking and alcohol consumption showed that the latter observation was stronger for never smokers and those with limited alcohol intake.

Selenium is required for normal thyroid function, but its link with the balance of sex steroid hormone levels remains to be defined (12, 20). While the studies by Zengt et al (20) and Rotter et al

(12) did not find any associations, a study investigating the association between serum levels of testosterone and selenium in infertile men attending a fertility clinic in South-East Nigeria, found a strong positive association (21). However, in the context of development of cancer, our finding of an inverse association between selenium and estradiol is in line with previous animal studies for breast cancer (22). Lee et al. investigated the effect of selenium on estrogen receptor (ER) expression and activation using methylselenic acid (MSA), an active form of selenium in human breast cancer cells (22). Selenium was found to decrease expression of ERalpha mRNA and protein and increased ERbeta mRNA expression in this in vitro study. This differential regulation of ERalpha and ERbeta in breast cancer cells has been proposed as a novel mechanism of selenium action in the context of breast cancer prevention (22). In the context of prostate cancer, the mechanism of action remains to be determined. Only one study has investigated the association between selenium and estradiol in the context of prostate cancer, but no association was found (23). However, several experimental studies support the observation that estrogens might play a role in prostatic carcinogenesis (24, 25). Even though we observed an inverse association between selenium and estradiol across racial/ethnic groups in NHANES, the higher estradiol concentration in non-Hispanic black men compared to non-Hispanic white men supports the known higher risk of prostate cancer in black men (14). The findings for the stratifications by smoking behavior, alcohol consumption and age require further biological studies as it is unclear why the associations were stronger in the non-smokers, those with limited alcohol consumption, and those of older age.

This is one of the largest studies assessing the concentrations of sex steroid hormones in relation to selenium. The sampling design of NHANES makes our findings applicable to the US population of civilian non-institutionalized men aged 20 years and older. We were also able to adjust for a wide range of potential confounding factors including lifestyle factors and race/ethnicity. As our study is a cross-sectional evaluation of the link between sex steroid hormones and selenium and it was not possible to assess temporality. Studies on sex steroid hormones are limited by high level of the

intersubject and intrasubject variability in the production, circulating levels, and metabolic clearance rates of steroid hormones resulting from changes in the diurnal rhythm. Here, we aimed to minimize the influence of variation resulting from diurnal production of the hormones by selecting participants with morning samples. Another limitation of this study is the lack of information on selenium concentrations in toenails, as the association with prostate cancer risk has been shown to vary based on selenium measurements (2). The data collection prior to implementation of PSA testing may also have resulted in the lack of prostate cancer diagnosis for some men – though these are though to be indolent cancers (26).

Conclusion

This cross-sectional study suggests that a possible mechanism by which selenium may be protective for prostate cancer is related to estrogen. Further studies of longitudinal measurements of serum and toenail selenium in relation to serum measurements of sex steroid hormones are needed. These studies would also benefit from having more detailed data on PSA measurements as well prostate biopsy tissue.

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Table 1. Age adjusted participant characteristics* by quartile of serum selenium.

	Quartile 1 <112 ng/mL	Quartile 2 112-122 ng/mL	Quartile 3 122-133 ng/mL	Quartile 4 ≥133 ng/mL
Age, years	45.46 (0.38)	46.02 (0.46)	45.63 (0.50)	45.32 (0.47)
Race – Ethnicity (%)				
Non-Hispanic white	76.04	75.08	80.51	79.97
Non-Hispanic black	15.08	13.46	5.71	5.73
Mexican-American	4.74	5.91	4.33	3.81
Other	4.12	5.56	9.45	10.48
Cigarette smoking (%)				
Never	27.11	29.28	36.26	39.94
Former	26.84	28.94	31.98	35.55
Current	46.16	41.79	31.77	24.51
Serum cotinine [(27)], ng/mL (%)	158.36 (14.77)	100.04 (10.96)	71.84 (9.24)	85.41 (7.74)
<3.08	46.26	51.68	61.21	64.53
3.08-15	1.44	3.73	3.43	1.44
≥15	52.30	44.60	35.36	34.03
Income <$20k, %	38.95	31.38	25.56	26.40
Alcohol consumption (%)				
Never	43.40	31.01	27.33	26.06
Up to once a week	15.47	19.50	20.21	14.50
2-3 times a week	15.71	14.83	18.69	18.33
4-6 times a week	9.32	18.61	18.03	19.82
Daily or more	16.20	16.05	15.44	21.30
Vigorous physical activity (%)	14.95	11.63	14.69	15.81
Percent body fat (%)	22.50 (1.09)	21.79 (0.59)	21.97 (0.76)	21.02 (0.55)

*Mean (standard error) or percentage

Table 2. Adjusted geometric means (95% confidence interval) by quartile of serum selenium

	Quartile 1 <112 ng/mL	Quartile 2 112-122 ng/mL	Quartile 3 122-133 ng/mL	Quartile 4 ≥133 ng/mL	p-trend
Total testosterone, ng/mL					
Age and race-ethnicity adjusted	5.09 (4.67-5.55)	5.03 (4.75-5.34)	5.14 (4.91-5.39)	5.02 (4.72-5/34)	0.901
Multivariable model 1†	4.94 (4.54-5.37)	4.95 (4.68-5.23)	5.17 (4.94-5.41)	5.15 (4.88-5.44)	0.122
Multivariable model 2‡	5.06 (4.65-5.50)	4.96 (4.73-5.20)	5.20 (4.00-5.41)	5.12 (4.86-5.39)	0.625
Total estradiol, pg/mL					
Age and race-ethnicity adjusted	39.32 (37.08-41.69)	36.42 (34.55-38.38)	34.59 (33.29-35.94)	34.28 (32.36-36.30)	0.002
Multivariable model 1†	38.19 (36.31-37.59)	35.84 (34.17-37.59)	34.88 (33.62-36.19)	35.18 (33.36-37.10)	0.038
Multivariable model 2‡	38.00 (36.03-40.08)	36.10 (34.44-37.84)	35.25 (34.06-36.49)	35.29 (33.53-37.14)	0.050
SHBG, nmol/L					
Age and race-ethnicity adjusted	36.61 (33.59-40.03)	33.98 (33.49-36.75)	34.40 (32.35-36.59)	34.83 (32.86-36.92)	0.550
Multivariable model 1†	35.96 (32.90-39.30)	33.77 (31.22-36.54)	34.54 (32.39-36.84)	35.20 (33.27-37.24)	0.945
Multivariable model 2‡	36.85 (34.37-39.51)	33.67 (31.43-36.06)	34.38 (32.49-36.59)	34.66 (32.99-36.42)	0.351
Androstanediol glucuronide, ng/mL					
Age and race-ethnicity adjusted	11.65 (10.07-13.49)	12.19 (11.43-13.01)	11.46 (10.64-12.34)	11.62 (11.03-12.24)	0.705
Multivariable model 1†	11.83 (10.24-13.66)	12.25 (11.50-13.04)	11.44(10.58-12.37)	11.53 (10.88-12.22)	0.490
Multivariable model 2‡	11.88 (10.30-13.70)	12.39 (11.58-13.25)	11.50 (10.65-12.43)	11.58 (10.91-12.29)	0.465
Estimated free testosterone, ng/mL					
Age and race-ethnicity adjusted	0.10 (0.09-0.11)	0.10 (0.10-0.11)	0.10. (0.10-0.11)	0.10 (0.09-0.11)	0.973
Multivariable model 1†	0.10 (0.09-0.11)	0.10 (0.10-0.10)	0.10. (0.10-0.11)	0.10 (0.10-0.11)	0.269
Multivariable model 2‡	0.10 (0.09-0.11)	0.10 (0.10-0.10)	0.10. (0.10-0.11)	0.10 (0.10-0.11)	0.390
Estimated free estradiol, pg/mL					
Age and race-ethnicity adjusted	1.00 (0.94-1.07)	0.94 (0.89-0.99)	0.88 (0.84-0.92)	0.87 (0.82-0.93)	0.003
Multivariable model 1†	0.97 (0.93-1.03)	0.93 (0.88-0.98)	0.89 (0.85-0.93)	0.89 (0.85-0.95)	0.023
Multivariable model 2‡	0.96 (0.92-1.01)	0.93 (0.88-0.98)	0.90 (0.86-0.94)	0.90 (0.85-0.95)	0.065
Estradiol*1000 / total testosterone					
Age and race-ethnicity adjusted	8.17 (7.45-8.96)	7.65 (7.09-8.24)	7.11 (6.74-7.51)	7.22 (6.66-7.82)	0.031
Multivariable model 1†	8.18 (7.42-9.02)	7.66 (7.09-8.27)	7.13 (6.76-7.53)	7.22 (6.71-7.77)	0.029
Multivariable model 2‡	7.95 (7.19-8.80)	7.70 (7.17-8.26)	7.17 (6.80-7.56)	7.29 (6.84-7.77)	0.086

SHBG= Sex hormone-binding globulin

†Adjusted for age and race-ethnicity, smoking status, and serum cotinine

‡Adjusted for variables in model 1 and household income, physical activity, alcohol consumption, and percent body fat

Table 3. Sensitivity and stratified analyses for the adjusted geometric mean of total estradiol (ng/mL) (95% confidence interval) by quartile of serum selenium. All models are adjusted for age, race-ethnicity, smoking status, serum cotinine, household income, physical activity, alcohol consumption, and percent body fat unless model is restricted or stratified by one of these covariates.

	Quartile 1 <112 ng/mL	Quartile 2 112-122 ng/mL	Quartile 3 122-133 ng/mL	Quartile 4 ≥133 ng/mL	p-trend
Race/Ethnicity stratification ($P_{interaction}$ = 0.910)					
Non-Hispanic white	37.77 (35.31-40.41)	35.56 (33.52-37.73)	34.56 (33.18-35.99)	35.06 (32.86-37.40)	0.145
Non-Hispanic black	43.10 (40.05-46.39)	40.78 (38.73-42.95)	40.30 (37.77-42.99)	39.25 (36.60-42.09)	0.121
Mexican-American	34.52 (33.66-35.41)	33.01 (30.69-35.51)	33.69 (30.88-36.76)	34.70 (32.11-37.49)	0.617
Other	35.91 (29.66-43.48)	37.39 (31.91-43.80)	38.07 (33.47-43.31)	34.42 (29.69-38.89)	0.637
Smoking ($P_{interaction}$ = 0.073)					
Never	37.31 (35.47-39.25)	33.16 (30.19-36.42)	31.93 (29.56-34.38)	31.65 (28.91-34.64)	0.002
Former	34.21 (30.54-38.32)	32.99 (30.54-38.32)	32.36 (30.23-34.65)	33.61 (31.72-35.62)	0.887
Current	37.31 (35.47-39.25)	33.16 (30.19-36.42)	31.93 (29.56-34.48)	31.65 (28.91-34.64)	0.579
Alcohol consumption($P_{interaction}$ = 0.017)					
Never	37.75 (35.67-39.96)	35.43 (32.96-38.09)	34.62 (32.83-36.50)	34.51 (31.48-37.82)	0.059
Up to once a week	38.42 (36.01-40.99)	36.79 (34.21-39.56)	34.87 (32.41-37.51)	33.34 (29.31-37.92)	0.086
≥2 times a week	38.18 (34.88-41.78)	36.42 (34.44-38.51)	35.41 (33.63-37.27)	36.29 (34.74-37.90)	0.345
Age stratification ($P_{interaction}$ = 0.612)					
20-40	38.81 (36.29-41.50)	37.85 (35.22-40.67)	37.07 (35.54-38,67)	36.70 (34.51-39.03)	0.287
40-60	36.32 (33.95-38.86)	35.29 (33.33-37.36)	33.80 (31.68-36.08)	34.31 (31.80-37.02)	0.211
60+	37.97 (34.81-41.43)	32.52 (29.85-35.43)	33.52 (30.83-36.43)	33.05 (30.37-35.97)	0.083

SHBG= Sex hormone-binding globulin